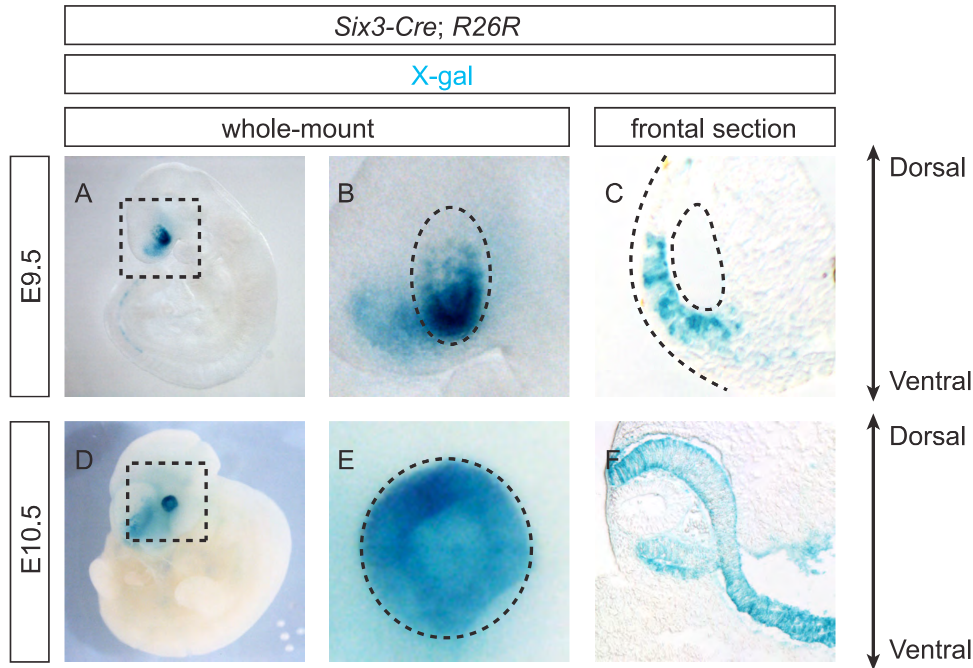

Fig. S1. Spatial and temporal mapping of *Six3-Cre* activity by the *R26R* reporter. (**A-C**) At E9.5, strong X-gal staining was first observed on the ventral side, but not on the dorsal side of the optic vesicle in *Six3-Cre;R26R* embryos. The boxed area in A is magnified in B. Frontal sections of the X-gal stained optic vesicle further demonstrate that *Six3-Cre* is initially active in the ventral side of the optic vesicle (C). (**D-F**) At E10.5, X-gal staining expands to the entire optic cup. The boxed area in D is enlarged in E. The X-gal stained eye in the D was further analyzed with frontal section (F).

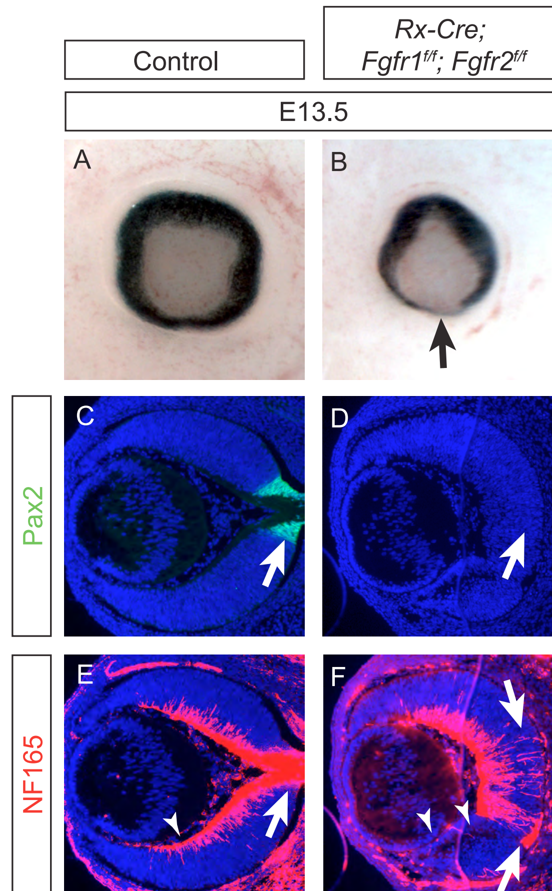

Fig. S2. Coloboma and optic disc dysgenesis in the *Rx-Cre;Fgfr1*$^{flox/flox}$*;Fgfr2*$^{flox/flox}$ mutants. (**A**,**B**) *Rx-Cre*-mediated deletion of *Fgfr1* and *Fgfr2* resulted in coloboma at E13.5 (arrow in B). (**C-F**) The *Rx-Cre;Fgfr1*$^{flox/flox}$*;Fgfr2*$^{flox/flox}$ mutants lose the Pax2-positive optic disc (arrow in D) and display misrouting of NF165-staining retinal ganglion cell axons (arrows in F). Neurogenesis defects are indicated by the loss of NF165 expression (arrowhead in F).

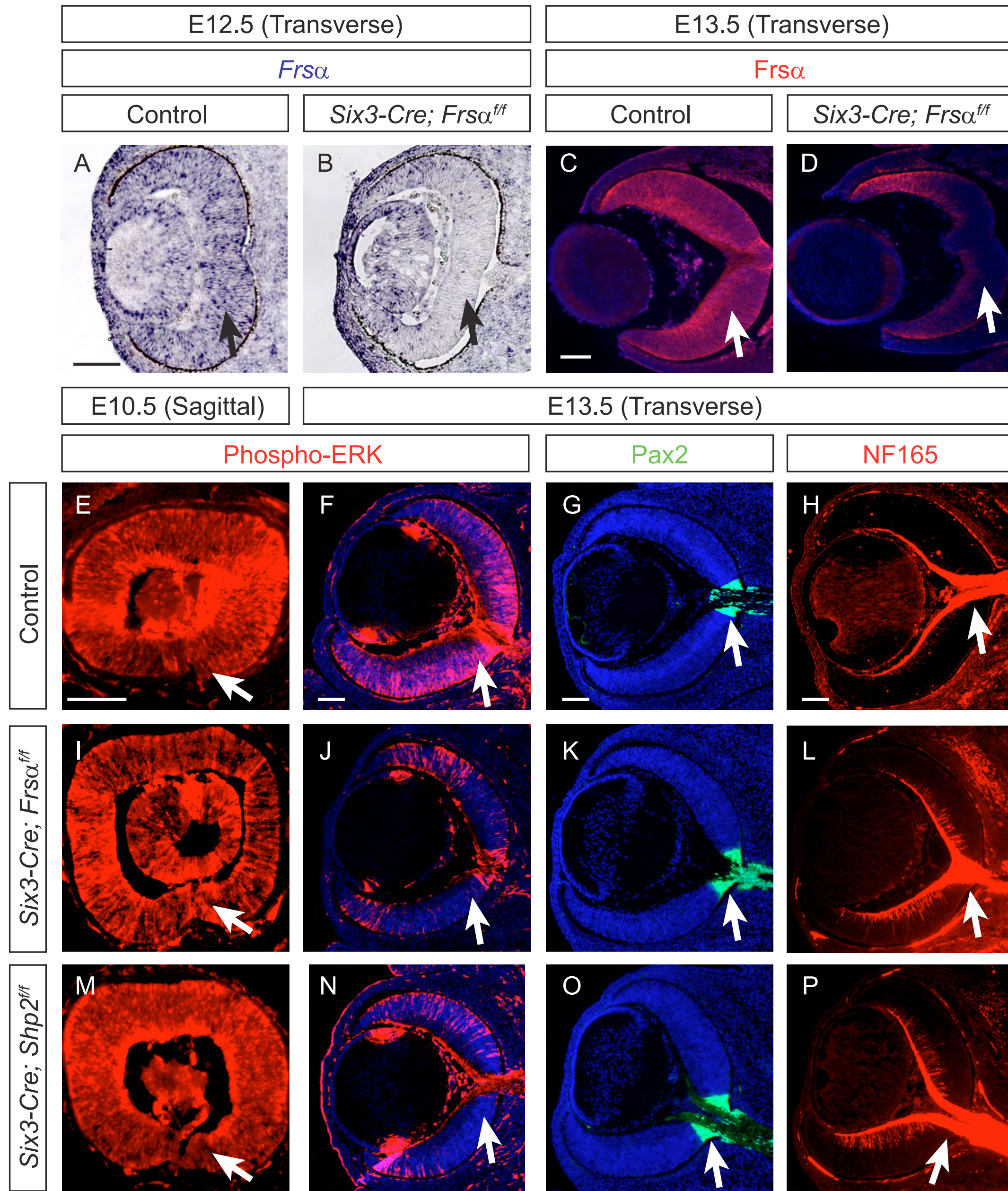

Fig. S3. Lack of ocular phenotypes in the *Six3-Cre; Frs2α*$^{flox/flox}$ and in the *Six3-Cre; Shp2*$^{flox/flox}$ mutants. (**A-D**) *Six3-Cre*-mediated ablation of *Frs2a* was confirmed in central retina by RNA *in situ* hybridization at E12.5 (A and B, arrows) and by immunostaining at E13.5 (C and D, arrows). (**E-P**) Deletion of *Frs2a* or *Shp2* alone reduced ERK phosphorylation in central retina at E13.5, but not at E10.5 (E,F,I,M,N, arrows). Normal optic disc formation as indicated by Pax2 staining and optic nerve genesis, as shown by NF165 labeling, was observed in both mutants (G,H,K,L,O,P, arrows). Scale bars: 100 µm.